S/N Unknown **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Jiri Snaidr	Examiner: Unknown
Serial No.:	Unknown	Group Art Unit: Unknown
Filed:	Herewith	Docket: 235.017US1
Title:	METHOD OF DETECTING MICROORGANISMS IN A SAMPLE (Continuation under 35 U.S.C. 111(a) of PCT/EP00/03989, filed May 4, 2000)	

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Prior to examination, please amend the above-identified continuation application as follows.

In the Specification

On page 1, after the title, please insert the following section:

--RELATED APPLICATIONS

This application is a continuation under 35 U.S.C. 111(a) of International Application No. PCT/EP00/03989 filed May 4, 2000 and published as WO 00/68421 on November 16, 2000, which claims priority under 35 U.S.C. 119 from German Application No. 199 21 281.3 filed May 7, 1999, and German Application No. 199 36 875.9 filed August 5, 1999, all of which applications are incorporated herein by reference.--

In the Claims

Please substitute the claim set in the appendix entitled Clean Version of Pending Claims for the previously pending claim set. The substitute claim set is intended to reflect amendment of claims 3, 5-10, 12, 13, 15, 16, 18, 20, 22, 24 and 25. The specific amendments to individual claims are detailed in the following marked up set of claims.

1. A method of detecting microorganisms in a sample by means of a nucleic acid probe comprising the following steps:

a) fixing the microorganisms contained in the sample;

b) incubating the fixed microorganisms with the detectable nucleic acid probe molecules;

c) removing nonhybridized nucleic acid probe molecules;

d) separating the hybridized nucleic acid probe molecules without using formamide and

e) detecting the separated nucleic acid probe molecules.

2. A method according to Claim 1, wherein the separated nucleic acid probe molecules in step e) are also quantified.

3. (AMENDED) A method according to Claim 1[or 2], wherein the separation solution used in step d) is selected from the group consisting of water, buffered water, DMSO and SSC.

4. A method according to Claim 3, wherein the separation solution is 0.001 - 1.0 M Tris/HC1, pH 9.0 +/- 2.0.

5. (AMENDED) A method according to Claim 3[or 4], wherein the separation solution is 0.01 M Tris/HC1, pH 9.0 +/- 2.0.

6. (AMENDED) A method according to [one of the preceding claims] Claim 1, wherein step d) is carried out at a temperature of 50 to 100 °C.

7. (AMENDED) A method according to [one of the preceding claims] Claim 1, wherein step d) is carried out at a temperature lower than 100 °C.

8. (AMENDED) A method according to [one of the preceding claims] Claim 1, wherein step d) is carried out at a temperature of approximately 80 °C.

9. (AMENDED) A method according to [one of the preceding claims] Claim 1, wherein the nucleic acid probe is complementary to a chromosomal or episomal DNA, an mRNA or rRNA of a microorganism to be detected.

10. (AMENDED) A method according to [one of the preceding claims] Claim 1, wherein the nucleic acid probe is covalently bonded to a detectable marker.

11. A method according to Claim 10, wherein the detectable marker is selected from the group of the following markers:

a) fluorescence markers,

b) chemoluminescence markers,

c) radioactive markers,

d) enzymatically active group,

e) haptene,

f) nucleic acid detectable by hybridization.

12. (AMENDED) A method according to [one of the preceding claims] Claim 1, wherein the microorganism is a single-cell microorganism.

13. (AMENDED) A method according to [one of the preceding claims] Claim 1, wherein the microorganism is a yeast, a bacterium, an alga or a fungus.

14. A method according to Claim 13, wherein the microorganism belongs to the genus *Salmonella*.

15. (AMENDED) A method according to [one of the preceding claims] Claim 1, wherein the sample is an environmental sample taken from water, soil or air.

16. (AMENDED) A method according to [one of Claims 1 through 14] Claim 1, wherein the sample is a food sample.

17. A method according to Claim 16, wherein the sample is taken from milk or milk products, drinking water, beverage, baked products or meat products.

18. (AMENDED) A method according to [one of Claims 1 through 14] Claim 1, wherein the sample is a medicinal sample.

19. A method according to Claim 18, wherein the sample is taken from tissue, secretions or fecal matter.

20. (AMENDED) A method according to [one of Claims 1 through 14] Claim 1, wherein the sample is taken from wastewater.

21. A method according to Claim 20, wherein the sample is taken from activated sludge, putrefactive sludge or anaerobic sludge.

22. (AMENDED) A method according to [one of Claims 1 through 14] Claim 1, wherein the sample is taken from a biofilm.

23. A method according to Claim 22, wherein the biofilm is taken from an industrial plant, is formed in purification of wastewater or is a naturally occurring biofilm.

24. (AMENDED) A method according to [one of Claims 1 through 14] Claim 1, wherein the sample is taken from a pharmaceutical or cosmetic product.

25. (AMENDED) A kit for carrying out the method according to [one of the preceding claims] Claim 1, containing

a) at least hybridization buffer,

b) at least one nucleic acid probe,

b1) for specific detection of a microorganism,

b2) for performing a negative control.

26. A kit according to Claim 25, containing at least one specific probe for detection of bacteria of the genus Salmonella.

27. A kit according to Claim 26, containing the nucleic acid probes

Salm63: 5'-TCGACTGACTTCAGCTCC-3'

and

NonSalm: 5'-GCTAACTACTTCTGGAGC-3'

or a nucleic acid probe that differs from Salm 63 and/or NonSalm by a deletion and/or an addition, whereby the ability of this probe to hybridize with Salmonella-specific nucleic acid is maintained, or a nucleic acid that can hybridize with the aforementioned nucleic acids.

Remarks

The specification has been amended to add information on related applications.

Claims 3, 5-10, 12, 13, 15, 16, 18, 20, 22, 24 and 25 have been amended to eliminate multiple dependencies; claims 1-27 are pending.

If there are any questions concerning this application, the Examiner is invited to telephone Applicant's undersigned attorney at (612-349-9587).

Respectfully submitted,

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"Express Mail" mailing label number: EL 873 858 817 US
Date of Deposit: November 7, 2001
This paper or fee is being deposited on the date indicated above with the United States Postal Service pursuant to 37 CFR 1.10, and is addressed to the Commissioner for Patents, Box Patent Application, Washington, D.C. 20231.

CLEAN VERSION OF PENDING CLAIMS

1. A method of detecting microorganisms in a sample by means of a nucleic acid probe comprising the following steps:

   a) fixing the microorganisms contained in the sample;

   b) incubating the fixed microorganisms with the detectable nucleic acid probe molecules;

   c) removing nonhybridized nucleic acid probe molecules;

   d) separating the hybridized nucleic acid probe molecules without using formamide and

   e) detecting the separated nucleic acid probe molecules.

2. A method according to Claim 1, wherein the separated nucleic acid probe molecules in step e) are also quantified.

3. (AMENDED) A method according to Claim 1, wherein the separation solution used in step d) is selected from the group consisting of water, buffered water, DMSO and SSC.

4. A method according to Claim 3, wherein the separation solution is 0.001 - 1.0 M Tris/HC1, pH 9.0 +/- 2.0.

5. (AMENDED) A method according to Claim 3, wherein the separation solution is 0.01 M Tris/HC1, pH 9.0 +/- 2.0.

6. (AMENDED) A method according to Claim 1, wherein step d) is carried out at a temperature of 50 to 100 °C.

7. (AMENDED) A method according to Claim 1, wherein step d) is carried out at a temperature lower than 100 °C.

8. (AMENDED) A method according to Claim 1, wherein step d) is carried out at a temperature of approximately 80 °C.

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14. A method according to Claim 13, wherein the microorganism belongs to the genus *Salmonella*.

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17. A method according to Claim 16, wherein the sample is taken from milk or milk products, drinking water, beverage, baked products or meat products.

18. (AMENDED) A method according to Claim 1, wherein the sample is a medicinal sample.

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